MONTANA STATE UNIVERSITY

The relationship of osmotic potential and carbohydrate during acclimation in crowns of two winter wheats differing in winterhardiness
by Kelly John Thomas

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University
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Abstract:
Winterhardiness is important for winter wheat (Triticum aestivum L.) production in Montana. It has been shown that osmotic potential and carbohydrate content of winter wheat crowns are good indicators of winterhardiness.

This research used controlled environment acclimation to study the relationship of osmotic potential and carbohydrate in the crowns of two varieties of winter wheat, 'Froid' and 'Brawny', with high and low winterhardiness, respectively.

Measurements of osmotic potential, percent crown . moisture, and concentrations of fructan, sucrose, glucose, fructose and total nonstructural carbohydrate (TNC) were taken each week beginning with the third and ending with the ninth week of acclimation following emergence.

Significant differences between varieties were detected for osmotic potential, percent crown moisture, fructan concentration and TNC concentration. Froid, the more winterhardy variety, maintained lower osmotic potentials, lower percent crown moisture and higher concentrations of fructan and TNC than the less winterhardy variety, Brawny.

In both varieties fructan, TNC and GFS (Glucose + Fructose + Sucrose) concentration increased while osmotic potential and percent crown moisture decreased during acclimation.

Multiple regression equations expressing osmotic potential as a function of several traits showed that fructan and GFS concentration accounted for 69% of the variation while TNG alone accounted for 66% of the variation. A single carbohydrate component, sucrose, accounted for 59% of the variation; however, much of the sucrose increase resulted from the breakdown of fructan.

Fructan was a major contributor to the total nonstructural carbohydrate pool accounting for up to 84% of TNC for the fourth and fifth weeks of acclimation after emergence.

The breakdown of fructan to shorter chain fructans and eventually to sucrose, glucose and fructose appears to be a major controlling force responsible for increased crown osmotic potential and therefore, increased winterhardiness in wheat.

THE RELATIONSHIP OF OSMOTIC POTENTIAL AND CARBOHYDRATE

DURING ACCLIMATION IN CROWNS OF TWO WINTER

WHEATS DIFFERING IN WINTERHARDINESS

by

Kelly John Thomas

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Kelly John Thomas

This thesis has been read by each member of the author's
graduate committee and has been found to be satisfactory
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ABSTRACT

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This research used controlled environment acclimation to study the relationship of osmotic potential and carbohydrate in the crowns of two varieties of winter wheat, 'Froid' and 'Brawny', with high and low winterhardiness, respectively.

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Fructan was a major contributor to the total nonstructural carbohydrate pool accounting for up to 84% of TNC for the fourth and fifth weeks of acclimation after emergence.

The breakdown of fructan to shorter chain fructans and eventually to sucrose, glucose and fructose appears to be a major controlling force responsible for increased crown osmotic potential and therefore, increased winterhardiness in wheat.

INTRODUCTION

Winterhardy varieties are very important for winter
wheat (Triticum aestivum L.) production in northern
latitudes. Much research is aimed at understanding the
mechanisms which allow the wheat plant to escape winter
injury (Pomeroy and Andrews, 1975; Gusta and Fowler, 1977;
Fowler and Gusta, 1977; Roberts, 1979; Andrews et al., 1984;
Roberts, 1985; Limin and Fowler, 1985).

Breeding winterhardy wheats is a difficult and time-
consuming process. The historical approach is to field
plant and evaluate winter injury. Due to variable weather,
non-differential winterkill--all plants live or all plants
die--is a common occurrence which makes it difficult to
determine winterhardiness of wheat cultivars.

Winterhardiness screening techniques should provide a
quick and precise indication of cultivar differences. These
techniques often involve cold stressing plants at controlled
temperatures and evaluating regrowth (Roberts and Grant,
1968; Metcalf et al., 1970; Pomeroy and Fowler, 1973).
Although these techniques work well, the greenhouse space
necessary to evaluate thousands of lines in a plant breeding
program is prohibitive.

Research completed at Montana State University suggested that winter wheat crown osmotic potential (DeNoma, 1989) and carbohydrate concentration (Wille, 1985) are good indicators of a cultivars winterhardiness. This research was initiated to determine the relationship between crown osmotic potential and carbohydrate concentration in acclimating winter wheat.

LITERATURE REVIEW

Temperature greatly influences cold hardening in winter wheat (<u>Triticum</u> <u>aestivum</u> L.). Winter cereals grown at temperatures warmer than 15 C are no more frost tolerant than spring cereals (Gusta et al., 1983). Generally, temperatures of less than 10 C are necessary to stimulate cold hardening in wheat (Olien, 1967; Gusta and Fowler, 1979); however, maximum cold hardening is not achieved unless the plants experience temperatures near 0 C (Olien, 1967; Pomeroy et al., 1975; Gusta et al., 1982). Although low temperature is the initiator of the hardening process in winter wheat, many physiological changes within the plant must occur to result in winterhardiness.

Crown moisture content of cereals has been linked to the ability to withstand low temperatures (Metcalf et al., 1970; Gusta and Fowler, 1976). Metcalf et al. (1970) found that slight increases in percent crown moisture of barley (<u>Hordeum</u> <u>vulgare</u> L.) and wheat diminished their ability to survive killing temperatures. Taylor and Olsen (1985 and 1986) found that low temperature was not necessarily the major factor contributing to winter injury in wheat. Well hardened winter wheat withstood crown temperatures as low as -20 C without appreciable injury. In field and controlled

environment studies, they found that dessication was a major cause of winter injury to wheat in Montana. Water relationships within the plant may play a large role in winterhardiness of winter cereals.

It has been suggested that frost tolerance in plants can be increased by artificially increasing the sugar content of the plant tissue. By infiltrating cabbage leaves (Brassica oleracea L.) with dextrose or fructose Levitt (1959) was able to increase frost tolerance by approximately 2 C. The actual results agreed very well with calculated values for increased frost hardiness on the basis of a purely osmotic effect (the decrease in osmotic potential caused a decrease in freezing point).

Russian researchers discovered that increases in winterhardiness of wheat placed in hardening conditions of 4 C TO -2 C closely paralleled substantial increases in oligosaccharide (short chain sugar) concentration (Bebenko and Gevorkyan, 1967). They concluded that oligosaccharides play a large role in protecting winter grains from low temperature injury.

Green (1972) increased cold hardiness in winter wheat by growing wheat seedlings in nutrient solution plus glucose, sucrose or mannitol. Measurements for frost tolerance, sugar accumulation and osmotic potential were recorded. Green found that although significant increases in frost tolerance occurred, he could not explain the increase due to

only an osmotic effect. All of the treated plants obtained
similar osmotic potentials (leaf osmotic potentials of
plants grown in glucose, sucrose and mannitol were -11.5, -
14.9 and -12.1 atmospheres, respectively), but the mannitol
treated plants were not nearly as cold tolerant as the
glucose or sucrose treated plants. Green deduced that some
other factor, possibly an oligosaccharide that accumulated
in the sugar treatments, but not the mannitol treatment had
imparted additional cold tolerance to the wheat plants by
other than an osmotic effect.

If osmotic effect can't completely explain the role of
sugars in winterhardiness of wheat, then what is the role of
these oligosaccharides? Olien (1965) isolated
polysaccharides (sugar polymers) from rye (Secale cereale
L.) crowns that appeared to interact with the ice crystal
interface and interfered with the freezing of water. He
concluded that these polysaccharides helped protect the rye
plant from low temperature injury not by reducing the
freezing point of the solution, as if the effect was purely
osmotic, but instead by interfering with the freezing
process causing irregular ice crystal formation.

Several ways sugars in the crown may affect winter
survival of wheat are by increased concentration of sugar
within the cellular fluid reducing the freezing point of the
crown tissue, by higher concentrations of sugar helping the
plant resist water loss due to dessication, and by oligo-

and polysaccharides interfering with ice crystal formation within the crown tissue.

The oligo- and polysaccharides are polymers of fructose. These fructans, as they are called, have the formula G-F-$(F)_n$ where G-F denotes a sucrosyl group and $(F)_n$ denotes one or more fructose units completing the polymer (Edelman and Jefford, 1968; Pollock, 1986). Molecules with a DP (degree of polymerization) of three or more are classified as fructans. Fructan is the major storage carbohydrate found in wheat and many other temperate season grasses (Wagner et al., 1983).

Most of the early work to determine the mechanism of fructan synthesis was done on Jerusalem artichoke (Helianthus tuberosus L.) due mainly to the large amounts of fructan containing tissue that can be obtained from relatively few plants (Edelman and Jefford, 1964; Edelman and Dickerson, 1966; Edelman and Jefford, 1968). More recently, work with asparagus (Asparagus officinalis L.) has helped to further explain enzyme characteristics in fructan accumulating plants (Shiomi and Izawa, 1980; Shiomi, 1981; Shiomi, 1982).

Edelman and Jefford (1968) outlined a general mechanism for control of fructan metabolism. They found that synthesis of fructans is controlled by two enzymes, SST and FFT, and depolymerization of the fructan molecule back to sucrose, glucose and fructose by another enzyme, FFH.

Sucrose:sucrose 1-fructosyltransferase (SST) was found to catalyze the following reaction creating a trisaccharide (DP = 3) from sucrose.

$$G-F + G-F \longrightarrow G-F-F + G$$

This enzyme uses sucrose as its only substrate and is inactive on the trisaccharide.

Fructan:fructan 1-fructosyltransferase (FFT) is highly specific for the transfer of fructosyl units (F) from one fructan to another fructan. It catalyzes the following reaction.

$$G-F-(F)_n + G-F-(F)_m \longleftrightarrow G-F-(F)_{n+1} + G-F-(F)_{m-1}$$
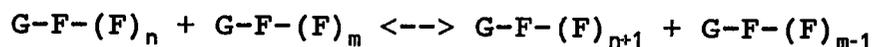
This enzyme works to increase the chain length or DP of fructans and can utilize any fructan as a substrate.

The breakdown or hydrolysis of fructan is accomplished by fructan 1-fructanohydrolase (FFH). This enzyme is also specific to fructan and catalyzes this reaction.

$$G-F-(F)_n \longrightarrow G-F-(F)_{n-1} + F$$

Although the major thrust of the work on fructan metabolism has been done on species such as Jerusalem artichoke, it is worth noting that SST activity has been found in barley (Wagner et al., 1986) (Wagner et al. 1983) and winter wheat (Housley and Daughtry, 1987).

Labeling patterns of fructans in winter wheat stems indicate that fructan synthesis in wheat is very similar to fructan synthesis in Jerusalem artichoke (Hogan and Hendrix, 1986).

Pollock and Ruggles (1976) found that in the temperate grass <u>Dactylis glomerata</u> L. fructan accumulation occurred in response to cool temperature (5 C) but not at warmer temperatures (20/15 C day/night). Several other reports of low temperature induction of fructan accumulation are available for barley (Sicher and Kremer, 1986), winter wheat (Wagner et al., 1983) and <u>Agropyron</u> ssp. (Chatterton et al., 1986). Fructan synthesis has been reported to occur at temperatures as low as 0 C (Pollock, 1986). In fact, SST remains very active at low temperatures. Wagner et al. (1983) found that SST isolated from wheat had an optimum activity at 28 C; however, half of the enzyme's activity was retained at 8 C. These results further emphasize that fructans may be involved in protecting plants from cold injury.

Wille (1985) in a study using eight varieties of winter wheat varying in winterhardiness, found a very high correlation (r = .707 to .915) between winterhardiness score and fructan concentration. The more winterhardy wheats tended to accumulate a higher amount of fructans than did less hardy wheats. Wille also determined that higher total nonstructural carbohydrate was associated with increased winterhardiness.

In contrast to the above study, Green and Ratzlaff (1975) found that concentrations of sucrose, glucose, fructose and raffinose were inversely related to

winterhardiness in winter wheat seedlings placed in fall hardening conditions.

Wagner et al. (1983) induced barley and wheat to accumulate fructans by removing their photosynthate sink and subjecting the plants to cold stress (5 C at night). Protoplasts of the barley leaves were then isolated and separated into their vacuolar and cytoplasmic components. They discovered that virtually all of the nonstructural carbohydrates (fructose, glucose, sucrose and fructan), along with SST and invertase activity, were associated with the vacuoles. Sucrose was the only sugar that approached equal concentration in both the cytoplasm and the vacuole. A similar situation was observed in the tubers of Jerusalem artichocke (Frehner et al., 1984). Wagner et al., (1983) concluded that storing fructans and other sugars in vacuoles is a good way of overcoming osmotic constraints of high accumulation of low molecular weight sugars. It is also ideally suited for osmoregulation of the plant during temperature or water stress. With an enzyme system such as discussed by Edelman and Jefford (1968), fructans can easily be depolymerized (converted to shorter chains, sucrose, glucose and fructose) decreasing the osmotic potential.

Osmoregulation is a very important factor in winterhardiness. DeNoma (1989) used crown osmotic potential as a criteria for ranking winter wheats according to winterhardiness score. He found high correlations between

crown osmotic potential and winterhardiness score of wheats
field sampled in December of 1983 and 1984 (r = .88 to .91).

MATERIALS AND METHODS

General Methods

Osmotic potential, several carbohydrates and percent
moisture were measured in crown tissue of two acclimating
varieties of winter wheat (Triticum aestivum L.), 'Froid'
and 'Brawny', which vary in winterhardiness (Froid = 81%
survival, Brawny = 39% survival, at 6 locations with
differential winter injury). The experiment was replicated
twice using a randomized complete block design for the first
4 weeks and a split-split plot design with two temperature
treatments as the main plots, varieties as the subplots and
sampling time (weeks of acclimation after emergence) as the
sub-subplot for the remaining five weeks of the experiment.

Two seeds of each variety were planted at a 3.0 to 3.5
cm depth in 65 cubic centimeter conetainers using 1:1:1
(sand:soil:peat) greenhouse mix. Two racks (400 cones) of
each variety were placed in an EGC M-13 customized growth
chamber fitted with high intensity lamps and increased
refrigeration capacity to simulate early fall field
acclimating conditions. Thinning after emergence left one
seedling per cone. Temperature conditions were split into
three periods outlined in Tables 1 and 2. Period I which
was carried out in the growth chamber for the first 4 weeks

of acclimation after emergence, simulated average fall acclimating conditions at Bozeman, Montana (Table 1). The growth chamber was unable to maintain temperatures low enough for the remaining acclimation periods; therefore, the plants were moved to modified chest freezers for periods II and III of acclimation. Temperature treatments were implemented during period II (fifth, sixth and seventh weeks of acclimation after emergence) by moving one rack of each variety from the growth chamber to the modified chest freezers at either +2 C or -2 C (Table 2). For Period III (eighth and ninth weeks of acclimation after emergence) the temperature in the freezers was lowered to -6 C (Table 2). Weekly random samples were taken beginning with the third week of acclimation after emergence for each variety and temperature.

Table 1. Weekly air temperature and photoperiod during period I in the plant growth chamber.

Weeks of Acclimation	Temperature (C)		Photoperiod (hrs)
	Day	Night	
1-2	20	7	13
3	15	-0.5	13
4	8	-3	13

Modifications on the freezers included replacing the
lids with a clear cryolite top and suspending fluorescent
lamps above to supply light for the plants. To assure no

Table 2.   Weekly air temperature and photoperiod during
          plant growth periods II and III for the low and
          high temperature regimes in modified chest
          freezers.

Period	Weeks of Acclimation	Low (C) Day	Night	High (C) Day	Night	Photoperiod (hrs)
II	5,6,7	-2	-2	+2	+2	13
III	8-9	-6	-6	-6	-6	13

drought or nutrient stress occurred, the plants were watered
and fertilized constantly while at above freezing
temperatures by submerging the lower 2 cm of the cone in
full strength Hoagland's solution. Photoperiod was set at
13 hours for the entire experiment with light levels of
1100 umol/m^2 sec for the first 4 weeks of acclimation after
emergence (in the growth chamber) and reduced to 40 umol/m^2
sec for the remaining five weeks in the freezers.

Nine plants were sampled from each variety on a weekly
basis beginning with the third week of acclimation. A
section of crown tissue was excised from each plant
extending from the base of the crown to one centimeter
above. The crowns were placed in microcentrifuge tubes and
heated in boiling water for 4 minutes to stop enzyme

activity. One crown was transferred immediately from the microcentrifuge tube to the thermocouple psychrometer sample chamber and the thermocouple junction inserted into the chamber. The chamber was sealed and placed on dry ice to freeze and disrupt the plant cell membranes. Readings for osmotic potential were taken using a microvoltmeter. The remaining crowns were frozen, vacuum dried and stored at -40 C for carbohydrate analysis later. Fresh and dry weights were taken on the crowns used for carbohydrate analysis to determine moisture percentage of the tissue.

Osmotic Potential Determination

Samples were prepared and measurements taken according to DeNoma (1989) with the following exceptions. Osmotic potentials were determined using J. R. D. Merrill model 84-2VC in situ thermocouple psychrometers and a Wescor HR-33T microvoltmeter. Temperature was controlled by submerging the psychrometers in water heated to 30 C in an AO Scientific Instruments shaking waterbath. The psychrometers were allowed to equilibrate in the water bath for a minimum of one hour prior to making measurements. One calibration curve was developed for each psychrometer using NaCl solutions as outlined by Weibe et al. (1971).

Carbohydrate Extraction

Dried crown tissue samples were ground with a Pitchford Manufacturing Corporation ball mill grinder. Fifty milligrams of ground plant material were extracted with 1.5 ml 80% v/v ethanol while agitating in a 60 C water bath, centrifuged at 1000 g for 5 minutes and the supernatant decanted and saved. The procedure was repeated on the pellet once more with 80% ethanol and twice with water. The supernatants were combined in a 10 ml beaker and placed in a 60 C constant temperature cabinet to drive off the alcohol. The remaining solution (approximately 1 ml) was frozen and thawed to help precipitate noncarbohydrate components in the solution and centrifuged to pellet out any undissolved material. The concentrated supernatant was washed with 25 ml of water through Dowex 50 and Dowex 1 cation and anion exchange resins, respectively, to remove charged molecules or particles. The solution was concentrated at 60 C to approximately 2.5 ml, divided into two equal portions, frozen and lyophilized. Acid hydrolysis of part of the lyophilized concentrate, as described below, resulted in the breakdown of all the carbohydrates to glucose and fructose. This information was used to determine the average degree of polynerization (AVGDP) of the fructan chains. The sample was dissolved in 250 ul of 0.1 N HCL and the solution heated in a waterbath at 85 C for 30 minutes. The solution was

neutralized with 125 ul of 0.2 N NaOH, washed with 20 ml of water through Dowex 50 and Dowex 1 exchange resins to remove the sodium and chloride ions and then concentrated to about one ml at 60 C, frozen and lyophilized.

Carbohydrate Analysis

Relative amounts of carbohydrate in the extracts were determined by high performance liquid chromatography (HPLC). Separation of the carbohydrate components (fructose, glucose, sucrose and fructans DP 3 to 6+) was accomplished using a Bio-Rad Aminex Carbohydrate HPX-42C Column heated to 85 C. The mobile phase was degassed double distilled deionized water at a flow rate of 0.5 ml/min. Carbohydrates were detected by a Waters R-401 Refractive Index Detector. Chromatographs corresponding to the carbohydrate were plotted and peak areas determined by a Shimadzu C-R3A Chromatopac integrator. Chromatograms and peak areas were obtained for both the hydrolyzed and extracted portions of the sample. The dry sample portions were dissolved in 0.5 ml of double distilled water, filtered with a 0.45 um syringe tip filter to remove small particles and 100 ul's of the sample injected into the sample loop by an ISCO ISIS HPLC Autoinjector.

Average degree of polymerization (AVGDP) of the fructan chains was calculated by first subtracting the fructose and glucose in the extracted plant sample from the glucose and

fructose in the hydrolyzed sample, computing the fructose to glucose ratio, and adding one to the ratio to account for the glucose unit in each chain.

Osmotic potential in wheat crowns is dependent on the total concentration of molecules in the cell sap (molality). Therefore, all concentrations of sugars were expressed as molality or as an estimation of molality.

Peak areas for sucrose, glucose and fructose were converted to concentration (molality) using standard curves and corrected for the mg of water per dry sample weight by the formula: molality x (500 mg sample soln)/(((mg fresh wt. - mgdry wt)/mg dry wt.) x 25 mg dry sample wt.) = corrected molality.

Since no standards are available for fructan, an estimate of the number of molecules of fructan in the sample was determined by dividing fructan peak area by the average degree of polymerization (AVGDP) of the fructan chains. This estimate of moles of fructan was corrected for the amount of water per dry sample weight with the formula: (Peak area/AVGDP)/(((mg fresh wt. - mg dry wt.)/mg dry wt.) x 25 mg dry sample wt.) = estimated fructan molecules/mg H_2O per sample dry wt. = Fructan Index (FI). Estimates of concentration for total nonstructural carbohydrates (TNC), fructan, sucrose, glucose and fructose were calculated using the formula: Peak area/(((mg fresh wt. - mg dry wt.)/mg dry

wt.) x 25 mg dry wt. per sample) = peak area(mV sec)/mg H_2O
per sample.

Thin Layer Chromatography

Thin layer chromatography (TLC) was used to qualify the
retention times of the fructan peaks from the HPLC analysis.
Precoated 20 x 20 cm silica gel glass plates (Keisilgel 60 F
254) were used to separate the fructans into individual
DP's. The sample, and sucrose, glucose and fructose
standards, were applied 1.5 cm from the bottom of the plate.
One hundred ml of the mobile phase (25 ml water : 50 ml 1-
butanol : 25 ml glacial acetic acid) was placed in the
chromatography tank along with the TLC plate. Six hours
were required for the solvent front to migrate to within 1
cm of the plate top. The plate was removed from the tank
and allowed to air dry. The standards and a portion of the
carbohydrate sample were developed by spraying with aniline-
diphenylamine Sigma spray reagent and heating at 85 C for 10
minutes. Three distinct bands corresponding to fructan
degrees of polymerization of 3, 4 and 5+ were scratched from
the plate and the fructan for each band extracted from the
silica gel with water. The extract was pushed through a
0.45 um syringe tip filter and run through the HPLC to
determine retention time. TLC samples were also quantified
for the degrees of polymerization of each band by Dr. N.
Jerry Chatterton, USDA Forage and Range Research Laboratory,

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Logan, Utah. Retention times for DP 3, 4, 5 and 6+ were
11.3, 9.7, 8.6 and 6.9 minutes respectively. Sucrose,
glucose and fructose eluted at 13.3, 16.0 and 19.3 minutes
respectively.

Statistical Analyses

Analysis of variance was computed for variables from
each week combined over runs, temperatures and varieties.
A combined analysis of variance was also computed for run,
temperature, variety and weeks of acclimation after
emergence. The individual varietal means and means for
weeks of acclimation were compared using the LSD calculated
with the appropriate mean square error from the ANOVA table.

Multiple regression analysis was used to examine the
relationships between osmotic potential and carbohydrates.

RESULTS AND DISCUSSION

Analyses of variance were computed for osmotic potential, fructan index (FI), glucose, fructose, sucrose, GFS (Glucose + Fructose + Sucrose), total nonstructural carbohydrate (TNC) and percent crown moisture (% CM). Varieties differed in period I for sucrose, fructose, GFS, TNC, and % CM with differences among weeks occurring for FI, sucrose, fructose, GFS, TNC and % CM (Table 3).

Table 3. Winter wheat crown tissue variety and week mean squares for eight variables from acclimation period I.

Source	df	Osmotic Potential		FI		Sucrose		Glucose		Fructose		GFS		TNC		% CM	
Run	1																
Var (V)	1	.114	ns	.575	ns	.0001	*	.000005	ns	.000006	*	.0002	*	31.8	*	27.3	*
Week (W)	1	.438	ns	.982	*	.0002	*	.000005	ns	.000015	*	.0001	*	46.3	**	28.9	*
V x W	1	.0009	ns	.006	ns	.00003	ns	.000041	ns	.000003	ns	.0002	ns	.62	ns	.37	ns
Resid	3	.136		.059		.000007		.000002		.0000005		.00002		1.16		1.07	

FI = Fructan Index (fructan moles/mg H_2O);
GFS = (Glucose + Fructose + Sucrose) (molality);
TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H_2O);
% CM = Percent Crown Moisture
*, ** = significant at $P < .05$ and $P < .01$, respectively.
ns = Not Significant.

Varieties also differed in periods II and III combined for osmotic potential, FI, GFS, TNC and % CM (Table 4). A significant variety x temperature interaction was detected for osmotic potential only (Table 4). This interaction is

explained by Froid, the more winterhardy variety reacting differently at the -2 C treatment than at the +2 C treatment. For periods II and III all the variables except fructan index (FI) and % CM differed significantly among weeks (Table 4).

Only FI and glucose differed significantly between the +2 C and the -2 C treatment implemented during period II (weeks of acclimation 5, 6 and 7) (Table 4). So few detectable differences may have resulted from reduced degrees of freedom due to the experimental design.

Table 4. Winter wheat crown tissue temperature, variety and week mean squares for eight variables during plant growth periods II and III.

Source	df	Osmotic Potential		FI		Sucrose		Glucose		Fructose		GFS		TNC		% CM	
Run	1																
Temp (T)	1	13.05	ns	6.34	*	.041	ns	.013	*	.006	ns	262.0	ns	.159	ns	13.1	ns
Err a	1	.244		.026		.002		.00004		.0001		7.2		.004		.566	
Var (V)	1	1.40	*	9.91	*	.004	ns	.00002	ns	.00008	ns	10.4	*	.004	*	174.0	*
T x V	1	1.97	*	.955	ns	.00001	ns	.00016	ns	.00002	ns	.18	ns	.0003	ns	16.0	ns
Err b	2	.026		.312		.0002		.00004		.000005		.494		.0002		7.05	
Week (W)	4	.774	*	.406	ns	.002	*	.0034	*	.007	*	37.6	**	.033	*	7.8	ns
T x W	4	.060	ns	.067	ns	.00002	ns	.0013	*	.0001	ns	1.96	ns	.002	ns	4.7	ns
V x W	4	.111	ns	.101	ns	.0001	ns	.00006	ns	.0002	ns	1.10	ns	.0009	ns	1.01	ns
TxVxW	4	.012	ns	.118	ns	.0001	ns	.00003	ns	.00007	ns	.34	ns	.0002	ns	.43	ns
Err c	16	.056		.236		.0003		.002		.0003		2.52		.002		.43	

FI = Fructan Index (fructan moles/mg H_2O);
GFS = (Glucose + Fructose + Sucrose) (molality);
TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H_2O);
% CM = Percent crown moisture.
*, ** = significant at $P < .05$ and $P < .01$, respectively.
ns = Not Significant.

Variety comparisons for eight variables over seven sampling weeks at the +2 C and -2 C temperature regimes are shown in Tables 5 and 6, respectively. Varieties differed for only 17 of 56 measurements taken at the +2 C regime (Table 5), whereas 28 of 56 measurements differentiated varieties at the -2 C regime (Table 6). Although the literature indicates that temperatures near 0 C are adequate to induce maximum hardening, it appears the -2 C temperature regime provided a better environment than the +2 C temperature regime for a differential varietal response. Furthermore, the -2 C temperature regime was more representative of the average acclimating conditions experienced by winter wheat in Bozeman, Montana. The major purpose of this study was to examine the relationship between osmotic potential and carbohydrates in the crowns of two winter wheats varying in winterhardiness. The +2 C and -2 C temperature treatments were included to assure a response. Since the best differential varietal response occurred at the -2 C temperature treatment, the bulk of the discussion is based on data from the -2 C temperature regime.

Osmotic Potential

Crown osmotic potential decreased significantly over the nine weeks of acclimation (Table 4; Figure 1). No significant differences between varieties occurred during